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Detection of *Monosporascus cannonballus* from melon plants using PCR

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Abstract: Root rot and vine decline caused by *Monosporascus cannonballus* is a major challenge for melon production world-wide. In recent years, a disease suggested to be related to this pathogen was observed 1-2 weeks prior to harvest in many melon production areas across Iran. In this study, melon plants with symptoms of chlorosis, wilting, decline and/or sudden death were collected from melon growing areas. Pieces of the roots with rot symptoms or discoloration were surface-sterilized and placed on PDA culture medium. DNA was extracted from the rest of the sterilized roots and used in polymerase chain reaction (PCR) using specific primers designed from ribosomal DNA of *M. cannonballus*. The pathogenicity of the fungus for 24 of its isolates was examined on a muskmelon genotype, Zard-e-Garmsar. In addition, the presence of *M. cannonballus* was tested on the symptomless melon plants at early growing stages as well as those inoculated with this pathogen using the specific primers. The presence of *M. cannonballus* was confirmed in 95 melon samples (63% of total samples tested) based on the morphological criteria of the isolated fungus and molecular techniques, where a unique band specific to this pathogen was amplified in diagnostic PCR. *M. cannonballus* was also detected in the roots of symptomless and inoculated melon plants as early as 2 days post-inoculation. This study demonstrated that *M. cannonballus* is the major causal organism for melon collapse in all sampling regions and that the pathogen is detectable in melon plants suspected of infection using molecular tools at early growth stages.

Keywords: Melon, *Monosporascus cannonballus*, PCR

Introduction

Root rot and vine decline of melon plants caused by *Monosporascus cannonballus* is an economically important disease world-wide. This pathogen has been reported from many arid and semi-arid regions including; Libya and India (Hawksworth and Ciccarone, 1978), Japan (Watanabe, 1979; Uematsu *et al*., 1985), Israel (Reuveni *et al*., 1983), Spain (Lobo Ruano, 1991), Tunisia (Martyn *et al*., 1994; Boughalleb *et al*., 2010), Taiwan (Tsay and Tung, 1995), Mexico (Martyn and Miller 1996), USA (Pollack and Uecker, 1974; Mertely *et al.*, 1991 and 1993; Bruton *et al*., 1995; Stanghellini *et al.*, 1996; Aegerter *et al*., 2000), Korea (Park *et al*., 1994), Guatemala (Bruton and Miller, 1997a), Honduras (Bruton and Miller, 1997b), Pakistan (Martyn 2002), Saudi Arabia (Karlatti *et al.*, 1997), Italy (Gennari *et al*., 1999), Brazil (Sales *et al.*, 2004) and Iran (Sarpeleh, 2008).

Root rot and vine decline is a generic term applied to a group of diseases with similar symptoms but different causal agents. The

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disease has been known as melon collapse (Garcia Jimenez *et al.*, 1994; Reuveni *et al.*, 1983), sudden wilt (Cohen *et al.*, 1996; Edelstein *et al.*, 1999; Eyal and Cohen, 1986; Pivonia *et al.*, 1997; Pivonia *et al.*, 1999), root rot (Kim *et al.*, 1995), vine decline (Cohen *et al.*, 1999), and root rot and vine decline (Martyn and Miller, 1996; Martyn *et al.*, 1994; Mertely *et al.*, 1991; Wolf and Miller 1998).

A number of fungi have been reported to be associated with melon collapse and the cause of collapse in many cases is unclear. *M. cannonballus* causes root rot and necrosis which results in reduced growth, progressive defoliation and partial or complete collapse of the plants towards the end of the season. Late season appearance of disease symptoms and similarities of the symptoms with those caused by other soil-borne melon fungal pathogens cause difficulties in disease management. In addition, no conidial stage has been detected for *M. cannonballus* and ascospores which are the only reliable fungal structure for the identification of this pathogen are produced after 3-4 weeks of incubation under certain circumstances (Pollack and Uecker 1974).

Early detection of plant pathogens including *M. cannonballus* is worthwhile as it grants enough time for growers to plan for disease control strategies. However, vine collapse is symptomatically detectable just prior to harvest and hence, the time to implement control procedures would be very limited.

Molecular tools provide fast and accurate detection for many economically important plant pathogens (Syarifah *et al.*, 2010; Henson and French, 1993; Michelmore and Hulbert 1987) including *M. cannonballus* (Lovic *et al.*, 1995; Pico *et al.*, 2008). Lovic *et al.*, (1995) designed 5 pairs of primers from ITS- region sequences of *M. cannonballus* in which a pair of the primers detected specifically *M. cannonballus* isolates. Pico *et al.*, (2008) designed three pairs of primers from the genomic rDNA (ITS1-5.8S-ITS2) regions in which one pair showed the highest sensitivity for detection of the pathogen in melon seedlings inoculated with *M. cannonballus*. The primers amplified a 112 bp segment of DNA extracted from 9 isolates of *M. cannonballus* as well as the DNA isolated from melon roots inoculated with the pathogen *in situ*. However, the potential of these specific primers to detect the pathogen from melon roots suspected of infection by *M. cannonballus* in field conditions needs further research.

The objective of this study was to examine the efficacy of these specific primers in early detection of *M. cannonballus* from symptomless melon plants at early growing stages as well as those suspected of being infected with *M. cannonballus.*

Material and Methods

Plant and fungal materials

During 2009-2010, muskmelon plants were collected from a field in Garmsar region, Iran at two growth stages: 8-10 leaf stage when the plants showed no disease symptoms and at maturity 10-20 d prior to harvest when the plants showed root rot and vine decline symptoms as described previously (Sarpeleh 2008). Melon plants (muskmelon, cantaloupe and watermelon) suspected to infection with *M. cannonballus* were also collected from several fields in Semnan, Fars, Yazd, Sistan and Baluchestan, Qazvin, Khorasan Razavi and Isfahan provinces during growing season between July and August 2010 (Table 1). Pieces of roots were used for *M. cannonballus* isolation and the rest of the roots were used for DNA extraction and detection of *M. cannonballus* using molecular tools (see below).

Primers

A pair of primers developed by Pico *et al.*, (2008) was used in the present study. The primers 5'-CTT ACC TAT GTT GCC TCG GCG-3' as forward and R: 5'-AAG AGT TTA GAT GGT CCA CCG G-3' as reverse were synthesized by Armin Shegarf Company (Tehran, Iran).

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Table 1 The regions and host plants from which *Monosporascus cannonballus* isolates were collected.

Isolate code	Sampling regions: Province	City	District	isolate code
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc251
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc252
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc253
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc254
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc257
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc258
cantaloupe	Isfahan	Kashan	Mohammad Abad	Mc259
cantaloupe	Isfahan	Kashan	Hossein Abad	Mc260
cantaloupe	Isfahan	Kashan	Hossein Abad	Mc262
cantaloupe	Isfahan	Kashan	Hossein Abad	Mc263
cantaloupe	Isfahan	Natanz	Galeh Goosheh	Mc267
muskmelon	Isfahan	Natanz	Galeh Goosheh	Mc269
muskmelon	Isfahan	Natanz	Galeh Goosheh	Mc271
cantaloupe	Isfahan	Ardestan	Mahbad	Mc273
cantaloupe	Isfahan	Ardestan	Mahbad	Mc275
cantaloupe	Isfahan	Ardestan	Mahbad	Mc278
cantaloupe	Isfahan	Ardestan	Mahbad	Mc281
cantaloupe	Isfahan	Natanz	Deh Abad	Mc282
cantaloupe	Isfahan	Natanz	Deh Abad	Mc283
cantaloupe	Isfahan	Natanz	Deh Abad	Mc284
cantaloupe	Isfahan	Natanz	Mogar	Mc286
cantaloupe	Isfahan	Kashan	Hossein Abad	Mc289
cantaloupe	Yazd	Meibod	Shams Abad	Mc301
muskmelon	Yazd	Meibod	Maryamd Abad	Mc306
muskmelon	Yazd	Meibod	Maryam Abad	Mc308
muskmelon	Yazd	Meibod	Hoork	Mc312
muskmelon	Fars	Darab	Ige	Mc342
muskmelon	Fars	Fasa	Zahed shahr	Mc350
muskmelon	Fars	Fasa	Zahed shahr	Mc354
muskmelon	Fars	Fasa	Zahed shahr	Mc357
muskmelon	Fars	Fasa	Zahed shahr	Mc358
muskmelon	Fars	Fasa	Zahed shahr	Mc359
muskmelon	Fars	Fasa	Zahed shahr	Mc360
muskmelon	Fars	Jahrom	Yousof Abad	Mc363
muskmelon	Fars	Jahrom	Yousof Abad	Mc364
muskmelon	Fars	Jahrom	Yousof Abad	Mc365
muskmelon	Fars	Jahrom	Yousof Abad	Mc366
muskmelon	Fars	Jahrom	Yousof Abad	Mc367
muskmelon	Fars	Jahrom	Yousof Abad	Mc368
muskmelon	Fars	Jahrom	Baba Arab	Mc369
muskmelon	Fars	Jahrom	Gotb Abad	Mc370
muskmelon	Fars	Jahrom	Gotb Abad	Mc371
muskmelon	Fars	Jahrom	Gotb Abad	Mc372
watermelon	Fars	Darab	Ige	Mc374
watermelon	Fars	Darab	Ige	Mc376
watermelon	Fars	Darab	Ige	Mc377
watermelon	Fars	Darab	Ige	Mc378
watermelon	Fars	Darab	Ige	Mc379
muskmelon	Semnan	Garmsar	Aradan	Mc419
muskmelon	Semnan	Garmsar	Aradan	Mc426
muskmelon	Semnan	Garmsar	Aradan	Mc423
muskmelon	Semnan	Garmsar	Aradan	Mc427
muskmelon	Semnan	Garmsar	Aradan	Mc447
muskmelon	Semnan	Garmsar	Aradan	Mc431
muskmelon	Semnan	Garmsar	Mashaie	Mc433
muskmelon	Semnan	Garmsar	khoosheh	Mc438
muskmelon	Semnan	Garmsar	khoosheh	Mc452
muskmelon	Semnan	Garmsar	khoosheh	Mc453
muskmelon	Semnan	Garmsar	khoosheh	Mc467
muskmelon	Khorasan Razavi	Taibad	-†	Mc471
muskmelon	Khorasan Razavi	Taibad	-	Mc473

Table 1 Continued

Isolate code	Sampling regions: Province	City	District	isolate code
muskmelon	Khorasan Razavi	Taibad	Tagi Abad	Mc475
muskmelon	Khorasan Razavi	Taibad	Tagi Abad	Mc476
muskmelon	Khorasan Razavi	Taibad	Tagi Abad	Mc478
muskmelon	Khorasan Razavi	Taibad	Tagi Abad	Mc479
muskmelon	Khorasan Razavi	Taibad	Ahmad Abad	Mc482
muskmelon	Khorasan Razavi	Taibad	Tagi Abad	Mc484
muskmelon	Khorasan Razavi	Mashad	Sarakhs Road	Mc492
muskmelon	Khorasan Razavi	Mashad	Sarakhs Road	Mc493
muskmelon	Khorasan Razavi	Torbat Jam	-	Mc510
muskmelon	Khorasan Razavi	Torbat Jam	-	Mc511
muskmelon	Khorasan Razavi	Taibad	-	Mc513
watermelon	Khorasan Razavi	Taibad	-	Mc514
muskmelon	Khorasan Razavi	Taibad	-	Mc517
muskmelon	Qazvin	Boein Zahra	-	Mc539
muskmelon	Qazvin	Boein Zahra	-	Mc541
muskmelon	Qazvin	Boein Zahra	-	Mc542
muskmelon	Qazvin	Boein Zahra	-	Mc543
muskmelon	Qazvin	Boein Zahra	-	Mc544
muskmelon	Qazvin	Boein Zahra	-	Mc550
muskmelon	Qazvin	Boein Zahra	-	Mc554
muskmelon	Qazvin	Boein Zahra	-	Mc555
muskmelon	Qazvin	Boein Zahra	-	Mc556
muskmelon	Qazvin	Boein Zahra	-	Mc557
muskmelon	Sistan&Balouchestan	Zabol	-	Mc559
muskmelon	Sistan&Balouchestan	Zabol	-	Mc560
muskmelon	Sistan&Balouchestan	Zabol	-	Mc561
muskmelon	Sistan&Balouchestan	Zabol	-	Mc562

†- : unknown district

Isolation of *M. cannonballus* from suspected plants

Surface sterilized root segments (3-5 mm), with or without brown spots/lesions were placed in Petri plates containing potato dextrose agar (PDA) amended with 250 ppm Ampicillin. After 4 days of incubation at 28 °C, hyphal tips were transferred to fresh Petri plates containing either PDA or corn meal agar (CMA) and incubated at room temperature (20-23 °C) for up to 40 days.

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Morphological and molecular identification of the isolates

The isolates were identified based on the morphology of peritecia, asci and ascospores formed on corn meal agar (Pollack and Uecker, 1974). To verify the morphological identification of *M. cannonballus*, six selected isolates were further investigated by molecular techniques using species-specific primers. DNA was extracted using previously described method with slight modification (Raeder and Broda 1985). Fresh fungal mycelium was grown in 100 ml Fries culture medium (Friis *et al.*, 1991) in 250 ml Erlenmeyer flasks for up to 3 weeks at 30 °C without agitation. Subsequently, the mycelium was ground with fine sand (SiO2; M = 60.06 g mol^{-1}) and liquid nitrogen. The ground mycelium (100 mg) was transferred into a centrifugation tube and extraction buffer (Tris-HCl pH 8.5 200 mM; NaCl 250 mM; EDTA 25 mM; SDS 0.5%) added. The mixture was homogenized gently and DNA of the samples was extracted using phenol and chloroform, and percipitated using cold isopropyl alcohol (Raeder and Broda, 1985). DNA was dissolved in 50 µl of double distilled water and kept at -20 °C until PCR amplification.

Pathogenicity testing of the isolates

Muskmelon seedlings (a local genotype named Zard-e-Garmsar) were inoculated with *M. cannonballus* isolates (Sarpeleh, 2008) and kept in a greenhouse at 28 ± 2 °C for up to 45 days. Inoculated plants were collected at 2, 4, 8, 16 and 32 days post-inoculation and used for re-isolation and detection of this pathogen using specific primers (see below).

In planta* PCR-based detection of *M. cannonballus

Ribosomal DNA was extracted from the roots of muskmelon plants either inoculated with *M. cannonballus* isolates (see above) or melon plants collected from different sampling regions (Table 1). After washing the roots of muskmelon plants to remove the soil, they were surface-sterilized for 2 minutes in sodium hypochlorite (1.5% available chlorine), and then washed twice in distilled water. The roots were then ground in liquid nitrogen using mortar and pestle. Total DNA was extracted from 1g aliquots of ground tissue, using modified Raeder and Broda method (1985). To this 7 ml of extraction buffer (as described above), 3 ml of phenol and 2 ml of chloroform was added shaken thoroughly then centrifuged at 4400 rpm for 35 minutes using a centrifuge (Hermle z320, Berthold Hermle GmbH & Co, Gosheim, Germany). Five µl RNAse (Vivantis inc., Swampscott, MA, USA) was added to the supernatant and the samples were kept at 37 °C for 30 minutes, equal amount of chloroform was added and then centrifuged for 30 minutes at 4400 rpm. The supernatant was collected in fresh tubes and the total DNA was percipitated using cold isopropanol. The pellet was washed twice in 70% ethanol then dissolved in 50 µl of double distilled water and kept at -20 °C until used for PCR amplification.

PCR analysis

Total DNA extracted from either pure fungal culture or the roots were used as a template in PCR using a thermocycler (Bio-Rad, USA). The PCR assay was performed according to the procedure of Pico *et al*., (2008) with some modifications, in a total volume of 25µl that contained PCR buffer (10 x) 2.5 µl; $MgCl_2$ (50 mM) 1.5 µl; forward and reverse primers (10 pmol) each 0.5 µl; *Taq* DNA polymerase (5 unit µl) 0.3 μl^{-1}; template DNA (25 ng μl^{-1}) 1 µl; dNTP mix (10 mM) 1 µl; double distilled H_20 17.7 µl. The PCR reaction was initiated with a pre-incubation at 50 °C for 2 minutes, and denaturing at 95 °C for 5 minutes, followed by 45 cycles of denaturing at 95 °C for 15 seconds and annealing and extension together at 60 °C for 1 minute per cycle, followed by a final extension step of 72 °C for 10 minutes.

Amplification product was separated by electrophoresis (75 V, 48 mamp, 25 min) in

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1.5% agarose gel in TAE buffer stained with 2% Ethidium bromide. The expected band was visualized with a UV transilluminator. The PCR product was then sent for sequencing (Ebn-e-Sina company, Tehran, Iran) to verify that the band obtained on agarose gel originated from the fungus (*M. cannonballus*) and not the plant template.

The sequences of nucleotides in the amplified segment were blasted (nucleotide blast) against *M. cannonballus* genome sequences available in national centre for biotechnology information (NCBI) databases (http://www.ncbi.nlm.nih.gov).

Results

Morphological and molecular identification of *M. cannonballus* isolates

Amongst 150 plant samples showing root rot and vine decline symptoms, 95 isolates produced globose perithecia after 25-30 days of incubation on CMA (Figure 1A). The perithecia were globose, smooth walled and 500−520 × 340−350 µm in diameter (Figure 1B). Asci were clavate, constricted at the base, unitunicate, thick walled, 93 × 46 µm in diameter and contained only one ascospore (Figure 1C). Ascospores were spherical, smooth, unicellular, thick-walled, 35−50 µm in diameter and hyaline at first, turning to dark brown at maturity (Figure 1D). No conidial stage was observed on PDA or CMA after one month of incubation at 25 °C and the fungus grew only as septate, hyaline hyphae 2−8 µm in diameter. Based on these characteristics, the isolates were identified as *Monosporascus cannonballus* Pollack & Uecker (1974).

A unique band 112 bp was observed when the PCR products of the fungal isolates were electrophoresed on agarose gel (Figure 2). Sequence alignment of the PCR products with nucleotide collection sequences available in NCBI databases revealed 98 % homology with ribosomal DNA sequences (ITS1) of *M. cannonballus*.

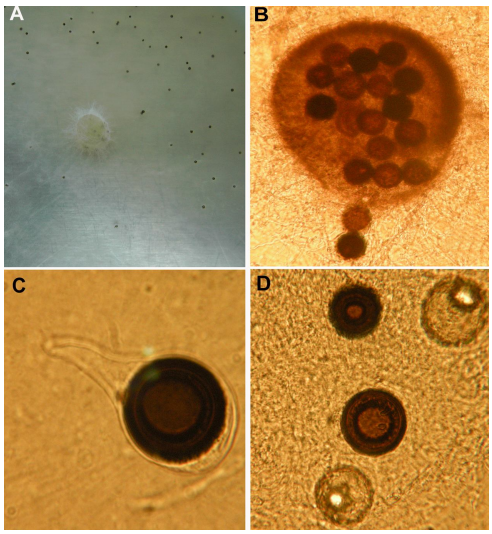

Figure 1 The fruiting bodies of *Monosporascus cannonballus*. Perithecia formed on corn meal agar after 25-30 days of incubation at 28 °C contained several asci (A & B) and one ascospore per ascus (C & D).

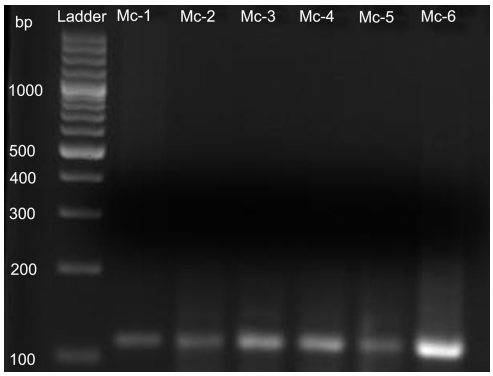

Figure 2 Polymerase chain reaction (PCR) product of genomic DNA extracted from different isolates of *Monosporascus cannonballus*. The isolates were collected from different provinces (Mc-1 = Isfahan, Mc-2 = Yazd, Mc-3 = Fars, Mc-4 = Semnan, Mc-5 = Qazvin, Mc-6 = Sistan and Baloochestan) used in PCR under condition described in the text and the PCR product visualized using agarose gel electrophoresis. All of the isolates tested on agarose gels produced their respective DNA fragment.

Pathogenicity testing of the isolates

The pathogenicity of 24 isolates was confirmed on a local genotype of muskmelon named Zard-e-Garmsar. The inoculated plants showed wilting, reduced growth and root rot symptoms 2-4 weeks post-inoculation (Figure 3). *M. cannonballus* was re-isolated from the roots of inoculated plants.

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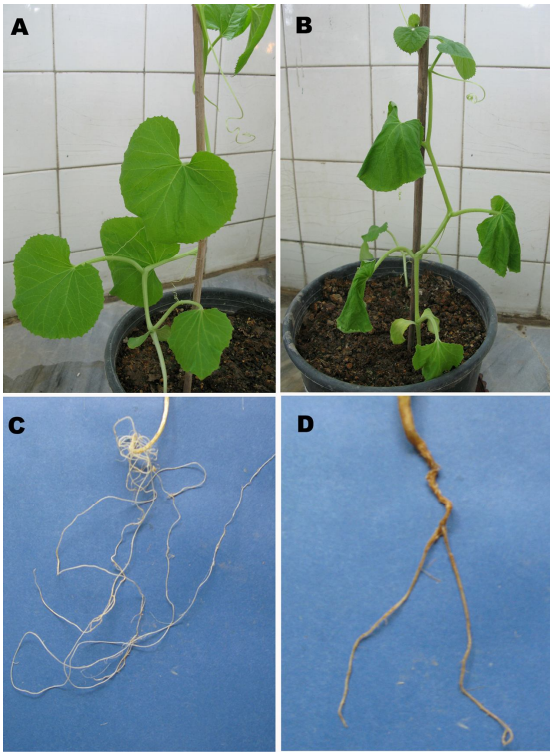

Figure 3 Pathogenicity testing of *Monosporascus cannonballus* isolates on muskmelon. The inoculums of 24 isolates of *M. cannonballus* collected from different places in Iran were prepared and used to inoculate a genotype of muskmelon named locally Zard-e-Garmsar. The mock inoculated (A & C) and inoculated plants (B & D) were kept at 28 ± 2 °C for up to 45 days under 16/8 light/dark photoperiod. The inoculated plants showed chlorosis and wilting in vine (B) and rotted feeder roots (D) at 2 weeks post-inoculation.

In planta PCR- based detection of *M. cannonballus*

M. cannonballus was detected using primers in inoculated roots as early as two days post-inoculation (Figure 4). This pathogen was also detected in melon plants grown under field conditions either in young symptomless muskmelon plants at early growing stages (8-10 leaf stage) or in melon plants 1-2 weeks prior to harvest showing root rot and vine decline symptoms. In PCR product, a single 112 bp fragment was observed in agarose gel and showed 98 % identity to *M. cannonballus* genome sequences available in NCBI databases (Figures 4 and 5).

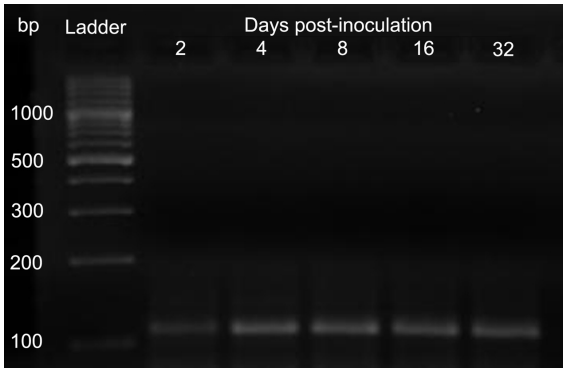

Figure 4 Detection of *Monosporascus cannonballus* in muskmelon roots cultivated in soil infested with 75 CFU of *M. cannonballus* g^{-1} of soil. Inoculated plants were analyzed at different days (2-32 days) after inoculation using polymerase chain reaction with primer pairs described in the text. A 112 base pair (bp) segment was amplified in all reactions.

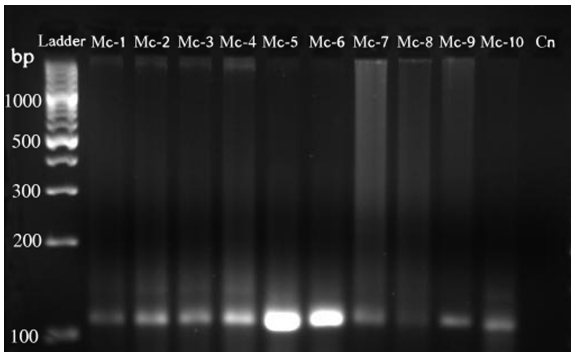

Figure 5 *In planta* detection of *Monosporascus cannonballus* using polymerase chain reaction (PCR). Melon plants suspected of infection with *M. cannonballus* were collected from different provinces of Iran and used to detect the fungus by PCR. A 112 bp fragment respective to *M. cannonballus* was amplified in all reactions. The correspondence of numbers with samples is as follows: Mc-1 = Semnan (symptomless muskmelon plant at 8-10 leaf stage), Mc-2 = Semnan, Mc-3 = Fars, Mc-4 = Yazd, Mc-5 = Sistan and Baloochestan, Mc-6 = Qazvin, Mc-7 = Fars, Mc-8 = Khorasan Razavi, Mc-9 = Isfahan, Mc-10 = Fars (watermelon), Cn = Negative control.

Discussion

This study demonstrated that specific primers developed for *M. cannonballus*, detect the Iranian isolates of this fungus and can be used

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to detect this pathogen in both symptomless and symptomatic melon plants grown under field conditions.

In recent decades melon collapse has become a major problem in many melon growing areas in Iran. Detection of disease based on symptom expression and identification of the causal organism based on morphological criteria are complicated while, early, rapid, and accurate identification of *M. cannonballus* in plant roots is essential to optimize strategies for disease management.

Species-specific primers have been developed for many phytopathogens including *M. cannonballus* (Lovic *et al*., 1995; Pico *et al.*, 2008). These primers detected nine isolates of *M. cannonballus* obtained from USA, Spain and Egypt (Pico *et al*., 2008). However the efficacy of the primers to detect Iranian isolates of *M. cannonballus* was unclear. In the present study, such primers were used to identify the Iranian isolates of *M. cannonballus*. 150 melon plants with root rot and vine decline symptoms were examined for *M. cannonballus*. Ninety five isolates were identified as *M. cannonballus* based on the morphology of fruiting bodies in synthetic culture media. Of these, 6 isolates were nominated (one isolate from each province) and employed to test the efficacy of the primers in detection of *M. cannonballus*. A 112 bp fragment was amplified in PCR reaction for all tested isolates and showed 98% homology with *M. cannonballus* gene sequences available in databases. This experiment demonstrated that primers developed for the identification of *M. cannonballus* (Lovic *et al*., 1995; Pico *et al.*, 2008) are able to identify the Iranian isolates of this pathogen.

In the second stage of the experiments, the primers were used to detect *M. cannonballus* in muskmelon plants in the early stages of disease establishment. *M. cannonballus* was detected in the roots of inoculated melon seedlings as early as 2 days after inoculation when no symptoms were yet evident. Similarly, *M. cannonballus* was detected in the roots of muskmelon plants at 8-10 leaf growing stage (20-30 d after sowing) while no symptoms of infection was visible in such plants and *M. cannonballus* could not be isolated from these samples when cultured on PDA. This reveals the high sensitivity of the specific primers in the early detection of *M. cannonballus* in young melon plants when conditions (Bruton *et al*., 1999; Pivonia *et al*., 2002) are not favorable for the development of infection and symptom expression.

The primers were shown to detect non-pathogenic isolates of *M. cannonballus*. In an independent experiment, the pathogenicity of three isolates of *M. cannonballus* with different colony morphology (yellowish color and fewer ascocarps in the culture media) was also tested in muskmelon plants (data not shown). These isolates showed no pathogenicity on melon plants while they were detected with the specific primers. Hypovirulent isolates of *M. cannonballus* have been reported to contain ds RNA and their growing features are different from those of the pathogenic isolates (Park *et al.*, 1996; Cluck *et al.*, 2009; Armengol *et al.*, 2011). Conversion of virulent to hypo-virulent and *vice versa* occurs in *M. cannonballus* isolates due to changes in temperature (Batten *et al.*, 2000; Martyn 2002). Such isolates exist in the roots of melon plants with reduced pathogenicity potential and may shift to wild type at high temperatures. Information on the haypo virulent populations of the fungus in the soil can be obtained using molecular techniques and is valuable in disease control strategies.

This study suggests that the specific primers developed for *M. cannonballus* can be employed as a forecasting tool using PCR technique, this however, requires further research. Regarding the cost and difficulty of DNA isolation from soil inhabiting micro-organisms such as *M. cannonballus*, it is suggested that the pathogen can be trapped to melon seedlings by random transplanting in the soil and detected in the seedling roots as early as 2 days post transplanting. The percentage of the infected seedlings can be used as an index of soil infestation with *M. cannonballus*.

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تشخیص *Monosporascus cannonballus* از بوته‌های طالبی و خربزه با استفاده از ابزار ملکولی

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چکیده: پوسیدگی ریشه و زوال بوته بر اثر *Monosporascus cannonballus* از مشکلات عمده تولید طالبی و خربزه در جهان می‌باشد. در سال‌های اخیر، عارضه‌ای در مناطق کشت این گیاهان در ایران، ۲-۱ هفته مانده به برداشت محصول مشاهده شده که به‌نظر می‌رسد بر اثر این بیمارگر حادث شده باشد. در این بررسی، بوته‌های طالبی و خربزه با علائم زردی، پژمردگی، زوال و یا مرگ ناگهانی از مناطق پرورش این گیاهان در ایران جمع‌آوری شدند. قطعاتی از ریشه با علائم پوسیدگی و یا تغییر رنگ، ضدعفونی سطحی شده و در محیط PDA کشت شدند. DNA از ریشه‌های استریل شده استخراج، و در حضور آغازگرهای اختصاصی *M. cannonballus* در واکنش زنجیره‌ای پلی‌مراز مورد استفاده قرار گرفت. یک نمونه متشکل از ۲۴ جدایه به‌طور تصادفی انتخاب و بیماری‌زایی آنها بر روی خربزه ژنوتیپ زرد گرمسار بررسی شد. علاوه بر این، حضور *M. cannonballus* در بافت‌های ریشه بوته‌های طالبی و خربزه فاقد علائم که در مراحل اولیه رشدی از مزارع جمع‌آوری شده و نیز بوته‌هایی که با این بیمارگر در گلخانه مایه‌زنی شده بودند، با استفاده از آغازگرهای اختصاصی مورد بررسی قرارگرفت. حضور *M. cannonballus* در ۹۵ نمونه گیاهی براساس شاخصه‌های مرفولوژیکی و نیز روشهای ملکولی تأیید شد. یک باند اختصاصی مربوط به *M. cannonballus* در محصول واکنش زنجیره‌ای پلی‌مراز بر روی ژل آگارز مشاهده گردید. *M. cannonballus* در ریشه گیاهان فاقد علائم و نیز ریشه بوته‌های مایه‌زنی شده خربزه با این بیمارگر تا ۲ روز پس از مایه‌زنی قابل تشخیص بود. این بررسی نشان داد که *M. cannonballus* از عوامل اصلی بوته میری پایان فصل طالبی و خربزه در ایران بوده و در بوته‌های مشکوک به آلودگی به این بیمارگر در مراحل اولیه آلودگی با استفاده از ابزار ملکولی قابل تشخیص است.

واژگان کلیدی: طالبی و خربزه، *Monosporascus cannonballus*، PCR

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